# Role of Inorganic Polyphosphate in Promoting Persistence of Mycobacteria

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## Abstract

In recent paper, He, et al., elegantly demonstrate the role of inorganic plyphospahte (polyP) in promoting persistence of Mycbacteria. Firstly, polyP affects the formation, morphology and ultramicrostructure of biofilms of Mycobacterium smegmatis by affecting the synthesis of short chain fatty acids. Furthermore, polyP regulates inflammatory factors expression through transition of macrophages from M1 to M2 type and promotes the survival of *M. smegmatis* in cells. Tuberculosis is still the main threat to human health. Persistence is an important cause of drug resistance and recurrence for *M. tuberculosis.*

**Keywords:** Polyphosphate; Mycobacteria; Hydrophobicity; Mutation

## Introduction

One of the important reasons for persistence is the formation of biofilm. In fact, *M. tuberculosis* grows in the form of biofilm *in vivo* and involves toxicity and drug resistance [1]. In addition, the formation of biofilm is closely related to the occurrence of infection, tissue necrosis, cavity formation, and the recurrence of tuberculosis [2].

Polyphosphate (polyP) is a linear molecule formed by high-energy phosphate bonds of phosphate radicals, widely present in the biological world [3]. The polyP kinases 1 (PPK1) is primarily responsible for maintaining bacterial polyP synthesis and Exopolyphosphatease (PPX) is responsible for the hydrolysis of polyP to inorganic phosphate [4]. They collectively regulate the dynamic homeostasis of intracellular polyP.

The dynamic homeostasis of polyP affects the formation of bacterial biofilm. In previous studies, we found that over-expression of PPX reduced intracellular polyP and affected the biofilm formation of *M. smegmatis* [5]. In He, et al., article, we found that the absence of polyP not only changed the morphology and ultramicrostructure of the biofilm, but also significantly reduced the survival ability of bacteria in macrophages [6].

## Methodology

To investigate the effect of polyP deficiency on the biofilm formation of *M. smegmatis*, He, et al., constructed *ppk1* mutant strains and complemented strains, and tested their intracellular polyP levels [6]. The results showed that the polyP levels in *ppk1* mutant strains were significantly lower than those in wild and complemented strains [6]. *ppk1* mutant strains not only exhibited attenuated biofilm formation ability, but also showed significant differences in biofilm morphology and ultramicrostructure compared to wild strains [6]. The initiation of biofilm formation involving interactions between cells or solid surfaces, once sufficient cells aggregate, extracellular matrix is generated to form biofilms. Mycobacteria does not have a special cell surface adsorption structure, so the components on the cell wall surface mediate the interaction process between bacteria or solid surfaces [5]. The lipids outside the cell wall endow the cell surface with hydrophobicity, thereby promoting the interaction between bacteria or solid surfaces and determining the initial stage of biofilm formation. Therefore, He, et al., analyzed the lipids (fatty acids) levels of *ppk1* mutant strains and found that the levels of various short chain fatty acids in the mutant strains were significantly reduced, indicating that changes in fatty acid levels are related to biofilm formation, morphology, and ultramicrostructure [6]. Consistent with the changes in the lipid composition of the cell wall, *ppk1* mutant strains also showed elevated sensitivity to antibiotics targeting the cell wall such as vancomycin.

The afore mentioned experimental evidences indicate that polyP deficiency leads to impaired biofilm formation, and its alteration in the morphology and ultramicrostructure of biofilm is related to changes in lipid composition on cell walls. How and what induce the formation of mycobacterial biofilms remains an important issue. The reasons for its impact on biofilm formation are not only related to the lipid composition of the cell wall surface, but also to the following two factors [7-14]. Firstly, the second messenger (p) ppGpp plays a very important role in biofilm formation. In Mycobacteria, (p) ppGpp is synthesized by *rel*, which promotes bacterial growth at low levels and biofilm formation at high levels [12]. Secondly, the metabolic state affects biofilm formation. The reduction of NADH induced biofilm formation, however, the aggregation of intracellular NADH could lead to significant changes in cell surface hydrophobicity and delayed biofilm formation [8,15]. Recently, Chakraborty, et al., elegantly reviewed the roles of lipids, (p)ppGpp and metabolic states in the biofilm formation of *M. smegmatis* [7]. However, it is currently unclear what the link between lipids, (p)ppGpp and metabolic state is in regulating the formation of biofilms.

## Results and Discussion

We propose that the metabolic state and (p)ppGpp are connected through polyP, thereby altering the biosynthesis of fatty acids and ultimately affecting the formation of biofilms (Figure 1). The reasons are as follows:

(1) PolyP regulates the expression of *rel* gene, leading to the synthesis of (p)ppGpp and ultimately affecting lipid metabolism. As shown in the Figure 1, polyP is located at a key node in the PPK1-polyP-MprAB-SigE-Rel signaling pathway. Phosphorylation and activation of the two component system MprAB by polyP induces the expression of *sigE* and *rel* genes in Mycobacteria, leading to an increase in (p)ppGpp [9,10]. (p)ppGpp regulates genes transcription and translation, involving various metabolic processes including lipid biosynthesis [11]. Although the specific mechanism is unknown, there are many examples for (p)ppGpp regulating lipid biosynthesis and affecting biofilm formation. For example, the inability to synthesize (p)ppGpp leads to changes in surface-related characteristics of *M. smegmatis*, such as impaired biofilm formation [12]. Moreover, the *rel* mutation of *M. smegmatis* reducd the biosynthesis of glycopeptides and polar lipids, resulting in altered cell surface characteristics [13]. Interestingly, The *rpoZ* gene deletion mutation of *M. smegmatis* resulted in attenuated biofilm formation and the inability to synthesize short chain fatty acids [14]. *ω* factor encoded by the *rpoZ* gene is the smallest subunit of RNA polymerase and can bind to ppGpp to regulate gene transcription [15]. Surprisingly, the biofilm morphology exhibited by the *rpoZ* mutant strains is very similar to that of the *ppk1* mutant strains. Scanning electron microscopy shows that the biofilm formed by the two mutant strains all lacks extracellular matrix, indicating that they may both affect biofilm formation through the (p)ppGpp [6,14].
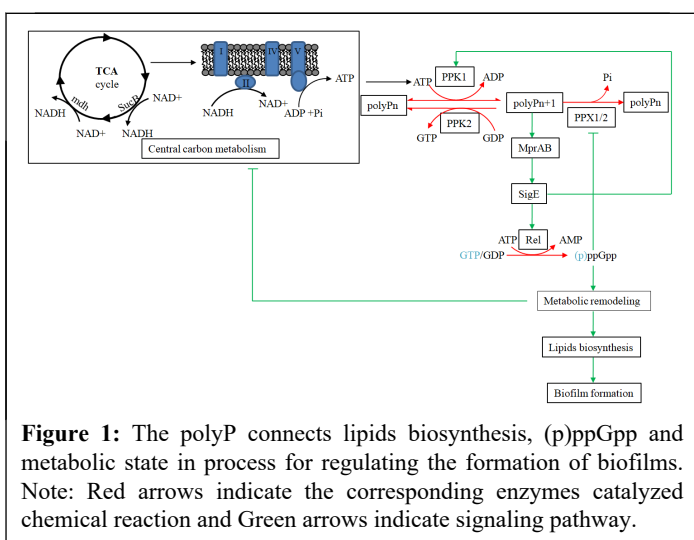


**Figure 1:** The polyP connects lipids biosynthesis, (p)ppGpp and metabolic state in process for regulating the formation of biofilms. Note: Red arrows indicate the corresponding enzymes catalyzed chemical reaction and Green arrows indicate signaling pathway.

The NADH generated by the TCA cycle enters into an electron transfer chain to produce ATP. *PPK1* uses ATP to synthesize polyP, which activates the two component system MprAB, thereby inducing the expression of *sigE*. Resultant SigE then induces the expression of rel, which catalyzes the synthesis of pppGpp and ppGpp with ATP and GTP/GDP. (p)ppGpp is a stringent response molecule that can lead to metabolic remodeling. Through this pathway, polyP connects metabolic states, (p)ppGpp, and NADH, thereby regulating the formation of biofilms.

(2) PolyP may regulate metabolic status of cells and alter the intracellular NADH/NAD+ ratio. The absence or aggregation of polyP in *M. tuberculosis* could lead to an increase or decrease in its sensitivity to isoniazid, and the aggregation of polyP in *M. smegmatis* also showed a decrease in sensitivity to isoniazid [16-20]. PolyP changes the sensitivity of Mycobacteria to INH, which may be related to alteration in the intracellular NADH/NAD+ ratio. A widely recognized superoxide production is the transfer of electrons from NADH through the electron transfer chain to oxygen [21]. The recent viewpoint is that antibiotics kill bacteria by producing superoxide through promoting NADH to quickly enter the electron transfer chain, accompanied with a decrease in the NADH/NAD+ ratio [22]. NADH is produced through the TCA cycle, so intermediate metabolites or related enzymes in the TCA cycle participate in the regulation of NADH. For example, *M. tuberculosis* up-regulated the expression of icl1 gene coding isocitrate lyase to increase the activity of the glyoxylate shunt and reduce the production of NADH in the TCA cycle, thereby reducing the production of reactive oxygen intermediates in the respiratory chain and then increasing resistance to the anti-tuberculosis drug such as INH [23]. Therefore, *M. tuberculosis* can increase resistance to anti-tuberculosis drugs through metabolic regulation such as remodeling of the TCA cycle (manifested as the aggregation of pyruvate, succinate, malate, and fumarate, while reduction of α-ketoglutarate) [23]. Interestingly, metabolomics analysis revealed that NAD+ levels and a large number of metabolites such as the acetyl-CoA, malate, and succinate significantly elevated in polyP-aggregated *M. tuberculosis* [24]. The changes in these metabolites in polyP-aggregated M. tuberculosis are highly consistent with those in *M. tuberculosis* induced by isoniazid, indicating that the aggregation of polyP may lead to remodeling of the TCA cycle to reduce the production of reactive oxygen species caused by NADH, and then leading to resistance to INH. The possible mechanism underlying polyP regulates metabolic status may be through the PPK1-polyP-MprAB-SigE-Rel signaling pathway. For example, the energy metabolism and redox homeostasis of the sigE mutation of *M. tuberculosis* are imbalanced, with central metabolic remodeling and reduced electron transfer chain activity [25]. Furthermore, polyP also affects intracellular ATP/GTP ratio. PolyP is a linear molecule formed by high-energy phosphate bonds between more than ten to hundreds of phosphate radicals. In the prokaryotic system, the synthesis and utilization of polyP are accomplished by *PPK1* and PPK2, respectively. *PPK1* is primarily responsible for reversible polyP synthesis with ATP, while PPK2 primarily utilizes polyP to produce GTP [26]. A rapid accumulation of polyP resulted in a drop in the intracellular ATP levels [27]. Moreover, GTP is required for the synthesis of (p)ppGpp and cell wall components of Mycobacteria.

Interestingly, polyP also affects the survival of *M. smegmatis* in macrophages, which is highly correlated with the persistence of Mycobacteria. He, et al., found that the survival of polyP-deficient mutant strains in vitro was not affected, but their survival ability in macrophages was significantly reduced [6]. By analyzing the expression levels of inflammatory factors in macrophages infected with ppk1 mutant strains, it was found that the expression levels of anti-inflammatory factor IL-10 in macrophages infected with *ppk1* mutant strains were significantly lower than those in macrophages infected with wild strains and complemented strains after 2 and 4 hours of infection [6]. After 24 hours of infection, the expression levels of pro-inflammatory factors IL-6 and TNF-α were significantly increased in macrophages infected with *ppk1* mutant strains, while the anti-inflammatory factor PPAR-γ was significantly reduced compared to

macrophages infected wild strains [6]. The fact that the absence of polyP alters the expression of inflammatory factors in macrophages infected with *M. smegmatis* suggests that polyP may alter the transition of macrophages from M1 to M2, thereby promoting bacterial survival within macrophages. Similarly, Rijal et al. reported that polyP secreted by *M. tuberculosis* increased the survival ability of its in human macrophages [28]. Moreover, the aggregation of polyP by *M. tuberculosis* increased the expression of IL-2 and IL-10 in infected macrophages. These results indicate that the polyP of Mycobacteria regulates the transition of macrophages from M1 to M2, thereby increasing the persistence of bacteria in macrophages.

## Conclusion

In summary, polyP affects the persistence of Mycobacteria. The polyP synthase PPK may serve as a potential drug target to reduce bacterial persistence, thereby reducing treatment time for tuberculosis and reducing the development of drug resistance.

## Acknowledgments

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